AD NUMBER

AD843455

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Foreign Government Information; 06 DEC 1967. Other requests shall be referred to Commanding Officer, Department of The Army, Fort Detrick, Attn: Technical Release Branch/TIO, Frederick, MD 21701.

AUTHORITY

SMUFD, D/A ltr, 15 Feb 1972

AD843455

TRANSLATION NO. 3073

DATE: 6 November 1968

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

NOV 21 1968

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/ TID, Frederick, Maryland 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

MULTIPLICATION AND VIRULENCE OF SEPTICEMIC AND BLOOD PATHOGENS IN THE ORGANISM OF INFECTED ANIMALS AND IN THEIR CARCASSES:

REPORT VI. STUDY OF A MODEL OF EXPERIMENTAL INOCULATIONS WITH *Listeria monocytogenes*

Izvestiya na Mikrobiologicheskiya Institut na B"lgarska Akademiya na Naukite (Bulletin of the Institute of Microbiology of the Bulgarian Academy of Sciences), Vol. 17, 1965, pages 135-141.

Al. Toshkov, D. Velyanov and Iv. Kaloyanov

The results of our research on the multiplication and virulence of certain septicemic and blood pathogens were reported in a series of preceding articles [1, 2, 3, 4, 5]. The results of our research on the multiplication and changes in the virulence of *Listeria monocytogenes*, set forth in the present article, are a direct follow-up of these.

Listeria monocytogenes is of interest as an object of study in the sense of our preceding research not only from the viewpoint of epidemiology and epizootiology, but also from the viewpoint of the doctrine of a natural focus of infectious diseases.

As is known, *List. monocytogenes* is pathogenic not only for man but also for a large number of domestic and wild animals. As a result, it must be emphasized, the cannibalism widely prevalent among the rodents and carnivores living in freedom in nature represents a significantly important link

in the epizootiology and natural focus of listeriosis. This being the case, any postmortal multiplication and rise in the virulence of List. monocytogenes would constitute an exceptionally important condition assuring the ready reinfection of wild animals in nature.

Materials and Methods

We worked accoróing to the technique described in some of the preceding articles [2, 4], using the guinea pig as experimental animal. The guinea pigs, each inoculated intraperitoneally with 1 ml of 24-hour broth culture of List. monocytogenes, died on the 4th-5th day.

We made the determination of the virulence of cultures, isolated at varying periods after death from the heart's blood of the experimental animals, on white mice as in the preceding works [1, 2, 3, 4, 5].

Three groups of experiments were conducted: Group I -- the carcasses of six guinea pigs that died after experimental inoculation, kept at a temperature of 28° C; Group II -- carcasses of five guinea pigs, kept at a temperature of 16° C; and Group III -- carcasses of six guinea pigs, kept in the refrigerator at a temperature from 0 to 4° C.

Results and Discussion

Table 1 gives the average results of the first group of experiments. From the data it can be seen that at the moment of death and at the 5th and 9th hour thereafter no Listeria are discovered at all despite 900X magnification of the field of view. At the 24th hour 1 or 2 bacteria are ascertained across each of several fields, while in the preparations made 36 and 48 hours afterwards no Listeria are discovered.

These data show that the bacteriemic titer of List. monocytogenes in the guinea pig before death is not high enough for the antibacterial properties of the blood to be overcome, and for this reason no postmortal multiplication of Listeria is observed in contrast to what was observed in the case of a number of pathogens in our preceding works [1, 2, 3, 4].

The microscopic findings in the liver and spleen at the moment of death show from 0 to 1-2 Listeria across a few fields, i. e. the findings are about the same as in the blood. Thereafter the number of bacteria increases ten-fold, to read 100-150 bacteria on the field of view at the 48th hour. It

merits mention that List. monocytogenes shows a definite tendency to multiply in nidi of 20-50 bacteria or more.

Table 1

RATE AND EXTENT OF POSTMORTAL MULTIPLICATION OF Listeria monocytogenes AT 28° C ON THE BASIS OF FINDINGS IN CARCASSES OF SIX GUINEA PIGS

	A Време след смъртта	B Кръв от сърцето (брой на листериите на зрително поле)	C Черен дроб (брой на листериите на зрително поле)	D Далак (брой на листериите на зрително поле)
1	Момент на смъртта	—	—	от 0 до 1—2 на няколко полета
2	5 ч.	—	от 1—2 до десетина	от 4—5 до десетина на поле
3	9 ч.	—	от 10 до 20	от 0 до гнезда от 20—25 бактерии през 1—2 полета
4	24 ч.	по 1—2 на няколко полета	от 5—6 до 20—30 на поле, тук-там гнезда от по 25—30 бактерии	от 15—20 до 40—50
5	36 ч.	—	от 4—5 до 50—60	10—15 (в някои препарати на гнезда по петдесетина през 2—3 полета)
6	48 ч.	—	от 10—12 до 100—150 на поле и в отделни полета гнезда от по няколко десетки до стотина листерии	

Keys (by columns):

A. Time after death
1. Moment of death
2. 5 hours
3. 9 hours
4. 24 hours
5. 36 hours
6. 48 hours

B. Blood from heart (number of Listeria in visual field)
4. 1-2 on several fields

C. Liver
2. 1-2 to 10
3. 10 to 20
4. 5-6 to 20-30 per field; here and there nidi of 25-30 bacteria
5. from 4-5 to 50-60
6. from 10-12 to 100-150 per field and in individual fields nidi of several tens to 100 List.

D. Spleen (number of Listeria in visual field)
1. from 0 to 1-2 on a few fields
2. from 4-5 to 10 per field
3. from 0 to nidi of 20-25 bacteria across 1-2 fields
4. from 15-20 to 40-50
5. 10-15 (in some preparations nidi of 50 each across 2-3 fields)

In contrast to the hundred- and thousand-fold multiplication of the pathogens used as models in our preceding works, the pace of multiplication here at the same temperature (28° C) is slower and for this reason the number of Listeria that have reproduced is smaller.

Table 2

RATE AND EXTENT OF POSTMORTAL MULTIPLICATION OF Listeria monocytogenes AT 16° C ON THE BASIS OF FINDINGS IN CARCASSES OF FIVE GUINEA PIGS

A Време след смъртта	B Кръв от сърцето (брой на листериите на зрително поле)	C Черен дроб (брой на листериите на зрително поле)	D Далак (брой на листериите на зрително поле)
1 Момент на смъртта	—	от 0 до 4—5 на зрително поле	от 3—4 през поле до 20—25 на зрително поле
2 5 ч.	—	10—12—15	5—12
3 9 ч.	от 1 до 7—9 през няколко полета	от 0 до 10—15	от 7—8 на поле до 20—30 бактерии на гнезда
4 24 ч.	—	от 10 до 18—20 на поле (тук-там на гнезда по 10—15)	от 10—15 до 70—80
5 36 ч.	—	от единичии до 100—130 на поле	от 50—60 до 200—300
6 48 ч.	по 2—3 през няколко полета	от 20—30 до 70—80	гнезда по 40—50, тук-там до натрупване от множество бактерии на поле

Keys (by columns):

A. Time after death
   1. Moment of death
   2. 5 hours
   3. 9 hours
   4. 24 hours
   5. 36 hours
   6. 48 hours

B. Blood from heart (number of Listeria on visual field)
   3. from 1 to 7-9 across several fields
   6. 2-3 across several fields

C. Liver (number of Listeria on visual field)
   1. from 0 to 4-5 on visual field
   3. from 0 to 10-15
   4. from 10 to 18-20 on field (here and there in nidi of 10-15
   5. from scattered ones to 100-130 on field
   6. from 20-30 to 70-80

D. Spleen (number of Listeria on visual field)
   1. from 3-4 across field to 20-25 on visual field
   3. from 7-8 on field to 20-30 bacteria in nidi
   4. from 10-15 to 70-80
   5. from 50-60 to 200-300
   6. Nidi of 40-50, here and there prior to accumulation of a great number of bacteria on field

Table 3

RATE AND EXTENT OF POSTMORTAL MULTIPLICATION OF *Listeria monocytogenes* AT 0 -- 4° C ON BASIS OF FINDINGS IN CARCASSES OF SIX GUINEA PIGS

A Време след смъртта	B Кръв от сърцето (брой на листериите на зрително поле)	C Черен дроб (брой на листериите на зрително поле)	D Далак (брой на листериите на зрително поле)
1 Момент на смъртта	0	от 1—2 до 3 (на няколко полета по 8—10—12)	1—2 до десетина (в един препарат огнище с 25—30 листерии на поле)
2 5 ч.	0	3—5 през поле	3—5
3 9 ч.	0	10—12—25 (на гнезда по 15—30)	5—15
4 24 ч.	0—1	15—20 (на гнезда по 35—40)	10—25 до 30
5 36 ч.	0	10—15—20	10—30 до 35 през зрително поле
6 48 ч.	0—1—2	15—18—20	—
7 72 ч.	0—2	50—60 (в един кръвоносен съд над 80)	40—50

Keys (by columns):

A. Time after death
   1. Moment of death
   2. 5 hours
   3. 9 hours
   4. 24 hours
   5. 36 hours
   6. 48 hours
   7. 72 hours

B. Blood from heart (number of *Listeria* on visual field)

C. Liver (number of *Listeria* on visual field)
   1. from 1-2 to 3 (on several fields 8-10-12)
   2. 3-5 across field
   3. 10-12-25 (in nidi of 15-30)
   4. 15-20 (in nidi of 35-40)
   7. 50-60 (in one blood vessel in excess of 80)

D. Spleen (number of *Listeria* on visual field)
   1. 1-2 to 10 (in one preparation focus with 25-30 *Listeria* on field
   4. 10-25 to 30
   5. 10-30 to 35 across visual field

The results of the second group of experiments, conducted at a temperature of 16° C, are set forth in Table 2. At this temperature, too, the results of the analysis of blood smears are negative with the exception of findings at the 9th and 48th hour where isolated bacteria are encountered across a few visual fields.

The findings in the liver and spleen at this lower temperature show no significant differences from those observed in the first group of experiments. In contrast to the considerable retardation in the tempo of multiplication with the reduction of temperature observed in the case of Past. pseudotuberculosis, Erys. rhusiopathiae and Bact. pyocyaneum, here this phenomenon is not observed to be so clearly pronounced. Even in the case of smears at the 36th and 48th hour ten-fold and greater multiplication of the Listeria is observed.

The results of the third group of experiments, conducted at a temperature of from 0 to 4° C, are given in Table 3. The data of microscopic findings in blood smears as well as in liver and spleen preparations show quite clearly that the postmortal multiplication of Listeria at refrigerating temperature proceeds considerably more slowly in comparison with the finding in the first two experimental groups at 28 and 16° C. It must be mentioned that the number of Listeria in the liver and spleen preparations made at the 72nd hour is already considerable and equals the number of bacteria by the 36th-48th hour at a temperature of 16° C. This is the result of comparatively more rapid postmortal multiplication of Listeria after the second 24-hour period.

In the third group of experiments the data from blood smears likewise coincide, especially during the later hours, with the results obtained in the first two groups of experiments. Consequently the antibacterial titer of the blood relative to List. monocytogenes is comparatively higher and cannot easily and quickly be overcome regardless of the considerable differences in temperature during the different groups of experiments.

Nor, it must be noted, -- just like our observations in the case of Past. pseudotuberculosis and Bact. pyocyaneum [2, 4], -- is any multiplication of septic microflora observed here as a counterbalance to that which was ascertained in the case of Erys. rhusiopathiae [3] -- exuberant multiplication of a large number of bacterial species penetrating into the blood and tissues of the digestive tract.

Table 4

VARIATIONS IN VIRULENCE OF Listeria monocytogenes IN CARCASSES OF GUINEA PIGS KEPT AT VARIOUS TEMPERATURES (INITIAL VIRULENCE OF STRAINS $3 \cdot 10^{-2}$)

A	B			
	Время след смъртта	Температура, при която се съхраняват труповете		
		28°C	16 °C	–4°C
1	Момент на смъртта	$5 \cdot 10^{-4}$	$5 \cdot 10^{-4}$	$5 \cdot 10^{-4}$
2	5 ч.	10^{-4}	$5 \cdot 10^{-3}$	$5 \cdot 10^{-4}$
3	10 ч.	—	$3 \cdot 10^{-3}$	10^{-3}
4	24 ч.	$3 \cdot 10^{-3}$	10^{-3}	$5 \cdot 10^{-3}$
5	36 ч.	$3 \cdot 10^{-3}$	—	10^{-3}
6	48 ч.	10^{-3}	$3 \cdot 10^{-3}$	$5 \cdot 10^{-3}$

Keys (by columns)

A. Time after death
   1. Moment of death
   2. 5 hours
   3. 10 hours
   4. 24 hours
   5. 36 hours
   6. 48 hours

B. Temperature at which carcasses were kept

Table 4 generalizes the titration results of changes in the virulence of Listeria monogytogenes in the carcasses of guinea pigs that died after experimental inoculation, kept at different temperatures. From the data for a temperature of 28° C it can be seen that at the moment of death virulence rose 60-fold in comparison with initial temperature. At the 5th hour it had risen 300-fold, to reach an increase of over 10,000-fold at the 24th hour, at which level it remained until the 36th hours. Thereafter, at the 48th hour, it fell sharply (hardly 30 times higher than initial temperature).

At a temperature of 16° C, virulence at the moment of death, just as above, rose 60-fold in comparison with initial virulence. Thereafter it showed a tendency towards a marked rise, mounting 600-fold at the 5th hour, 1000-fold at the 10th hour and attaining its maximum of 3000-fold at the 24th hour. Regardless of its considerable drop at the 48th hour, it remains 10 times greater than initial virulence.

As can be seen from the results given for a temperature from 0 to 4° C, the virulence of Listeria monocytogenes at this temperature increases more slowly during the first 24-hour period (600-fold at the 24th hour), reaching its maximum increase of 3000-fold at the 36th hour after death. At the 48th hour it drops to almost initial virulence.

Comparison of the data from the above three experiments reveals that the change in virulence of Listeria monocytogenes in the carcasses proceeds most rapidly and reaches the highest level at a temperature of 28° C. At a temperature of 16° C and at a temperature from 0 to 4° C virulence attains the same maximum of 3000-fold, but this rise at temperature 0-4° C is slower and is reached a little later, at the 36th hour after death.

In this regard List. monocytogenes proves similar to Past. pseudotuberculosis [2] and differs significantly from the systematically more akin Erys. rhusiopathiae, in whose case the temperature decrease has a sharp effect on the extent to which its virulence rises in the carcasses after death.

Like a few of the septicemic agents studied by us earlier [2, 3, 4], Listeria monocytogenes also manifests a well-pronounced capacity, as can be seen from the facts set forth above, to multiply in the carcasses of dead animals at a temperature from 0-4° C. This fact, just as the fact that the virulence of Listeria monocytogenes at this temperature attains, albeit more slowly, the same level as at a temperature of 16° C (up to 3000-fold in comparison with initial virulence), is of extraordinarily great significance and shows that in the case of listeriosis too carcasses have a most important role as a reservoir and a very dangerous source of infectious outbreak.

All these data, ascertained during our research on the multiplication and virulence of Listeria in the organism of infected animals and especially in their carcasses, place listeriosis in an entirely new light from the viewpoint of epizootiology and epidemiology and, above all, from the viewpoint of the doctrine of the natural focus of infectious diseases.

Conclusions

On the basis of the experiments here conducted the following conclusions can be drawn:

1. Parallel with the occurrence of comparatively more

moderate postmortal multiplication of Listeria monocytogenes in the tissues of animals that have died of experimental listeriosis, there takes place also a very great increase in the virulence of this microbe. In contrast to the tissues, no multiplication of Listeria is observed in the blood.

2. The different temperatures have more influence on the rate at which postmortal multiplication takes place than on the number of bacteria. At 28° C the number of Listeria increase 100-fold by the 48th hour, at a temperature of 16° C 30- to 40-fold by the 48th hour, while at refrigerating temperature the same level is barely reached by the 72nd hour.

3. The intensification of the virulence of Listeria monocytogenes which takes place simultaneously with the postmortal multiplication is likewise not directly dependent on temperature changes. At 28° C it reaches its maximum by the 24th to 36th hour (10,000-fold in comparison with initial virulence), while at a temperature of 16° C the increase in virulence reaches a maximum of 3,000-fold at the 24th hour. At a temperature of 0-4° C the rise of virulence takes place more slowly, and this same maximum is hardly reached at the 36th hour.

4. The great differences registered between passage virulence (during the disease) and postmortal virulence of Listeria monocytogenes emphasizes the great role of carcasses of animals that have died from listeriosis from the viewpoint of epizootiology, epidemiology and the doctrine of the natural focus of infectious diseases.

Submitted to press 26 November 1964.

BIBLIOGRAPHY

1. Toshkov, Al., As. Toshkov and G. Kamburov, "Multiplication and Virulence of Septicemic and Blood Pathogens in the Organism of Infected Animals and their Carcasses. Report I," Izvestiya na Mikrobiol. inst. na BAN (Bulletin of the Institute of Microbiology of the Bulgarian Academy of Sciences), 16, 1964.

2. Toshkov, Al., and D. Belyanov, "Multiplication and Virulence of Septicemic and Blood Pathogens in the Organism of Infected Animals and their Carcasses. Report II," Izvestiya na Mikrobiol. inst. na BAN (Bulletin of the Institute of Microbiology of the Bulgarian Academy of Sciences), 16, 1964.

3. Toshkov, Al., and Iv. Kaloyanov, "Multiplication and Virulence of Septicemic and Blood Pathogens in the Organism of Infected Animals and their Carcasses. Report III," Izvestiya na Mikrobiol. inst. na BAN (Bulletin of the Institute of Microbiology of the Bulgarian Academy of Sciences), 16, 1964.

4. Toshkov, Al., and D. Belyanov, "Multiplication and Virulence of Septicemic and Blood Pathogens in the Organism of Infected Animals and their Carcasses. Report IV," Izvestiya na Mikrobiol. inst. na BAN (Bulletin of the Institute of Microbiology of the Bulgarian Academy of Sciences), 16, 1964.

5. Toschkoff, Al., As. Toshkov, M. Radeva, I. Droumchev and Y. Karadzhov, "Further Studies on the Causes of Easy Infections during the Post-Mortem Examinations of Septic Cadavers" [English in source text], Comptes rendus de l'Académie bulgare des sciences (Reports of the Bulgarian Academy of Sciences), Vol. 18, No. 2, 1965.

SUMMARY

Follow-up studies were made of the multiplication and virulence of Listeria monocytogenes in the organism of experimentally inoculated guinea pigs and in their carcasses after death.

On the basis of these experiments it was established that simultaneously with a comparatively moderate postmortal multiplication of Listeria monocytogenes in the tissues there takes place as well an extraordinary intensification of its virulence. No multiplication is observed in the blood of experimental animals. It is noted that temperature changes affect the rate at which multiplication takes place and virulence increases after death. The increase of virulence in the carcass takes place in the following order: at 28° C 10,000-fold by the 24th-36th hour; at 18° C 3,000-fold by the 24th hour; and at 0-4° C 3,000-fold by the 36th hour.

The slight increase in virulence during the disease (up to 60-fold in comparison with initial virulence) and the sharp rise after death (up to 10,000-fold) emphasize the important role of carcasses as a significantly more dangerous reservoir and source of infectious outbreak than the organism of animals suffering from listeriosis.